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Effect of Ammonium Enrichment on Respiration, Zooxanthellar Densities, and Pigment Concentrations in Two Species of Hawaiian Corals[1]

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ABSTRACT: Small branch tips or "nubbins" of two species of Hawaiian corals, *Pocillopora damicornis* (Linnaeus) and *Montipora verrucosa* Vaughan, were exposed to four ammonium concentrations, ammonium-stripped (<2 μM), ambient ($\approx$2 μM), and two enriched (20 μM and 50 μM) in microcosm tanks. Nubbins represent replicates of a single coral colony. We examined the effect of ammonium enrichment on zooxanthellar densities, pigment concentrations, and respiration rates of the nubbins. Nubbins of both *P. damicornis* and *M. verrucosa* showed a trend of increased pigment concentration with elevated ammonium concentration. *Pocillopora damicornis* increased from 9.3 μg chlorophyll *a* cm^{-2} in the ammonium-stripped treatment to 24.8 μg cm^{-2} in the 50-μM ammonium treatment. Similarly, *M. verrucosa* increased from 1.9 to 19.4 μg chlorophyll *a* cm^{-2}. There were no significant differences in algal densities, pigment concentrations per cell, pigment ratios, or respiration rates.

CORAL REEFS DEVELOP in oligotrophic waters where the mutualistic association between coral animal host and endocellular microalgae (the zooxanthellae) enables corals to thrive in the ambient low nutrient concentrations (Muscatine and Porter 1977, Falkowski et al. 1993). During the last decades, increasing areas of coral reefs have been exposed to anthropogenic eutrophication (Davies 1990, Scott 1990). Nutrient concentrations are known to have impacts on coral physiology (Høegh-Guldberg and Smith 1989, Muscatine et al. 1989, Rahav et al. 1989, Dubinsky et al. 1990, Stambler et al. 1991). The response of a coral to any environmental perturbation may be related to physiological differences among species and colony morphologies, including differences between perforate and imperforate colonies.

The family Pocilloporidae has been the focus of many studies on the effects of light and nutrients on coral physiology. The Hawaiian pocilloporid *Pocillopora damicornis* (Linnaeus) is a finely branched coral with an imperforate skeleton. *Montipora verrucosa* Vaughan (Family Acroporidae) occurs in two morphologies, one platelike and one branched (used in this study), and has a perforate skeleton (Jokiel 1978).

These corals have been demonstrated to show species-specific responses to water motion (Jokiel 1978), with *P. damicornis* growing best in moderate water motion and *M. verrucosa* showing the best growth in low water motion. These corals also show different responses to changes in light regime. The perforate species, *M. verrucosa*, responded to changes in light levels with changes in the density of algal cells; the imperforate species, *P. damicornis*, responded with a change in pigment concentration per algal cell (Kinzie et al. 1984).

Small branch tips or "nubbins" of corals can be used to investigate the intra- and intercolony variation in physiological parameters (Davies 1989, 1991). The use of nubbins

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allows replication while optimizing use of available tank space. The results of this study can be compared with values for whole-colony performance. The aim of this study was to examine the effect of ammonium enrichment on nubbins of *P. damicornis* and *M. verrucosa*.

MATERIALS AND METHODS

Nubbin Preparation

Colonies of *P. damicornis* and *M. verrucosa* were collected at 1–3 m depth from several locations within Kaneohe Bay, Hawaii. Large colonies (10–20 cm diameter) were brought to the laboratory in buckets of seawater and held temporarily in tanks with running seawater. Each colony was broken into several nubbins containing one to three branches from the parent colony. Nubbins were ca. 2–3 cm in length. Only healthy nubbins completely covered with live tissue were used in these experiments. To hold nubbins in a vertical orientation, nubbins were glued into 4-cm-long sections of PVC tubing (1.5 cm diameter) using underwater epoxy. Nubbins acclimated for ca. 2 weeks in tanks with running seawater. The sections of PVC tubing fit into holes drilled in wooden racks for placement in the experimental tanks.

Nutrient Experiment

The nutrient experiment was carried out in eight white fiberglass tanks with a water volume of ca. 400 liters (1.15 by 1.15 by 0.27 m). Tanks were supplied with unfiltered running seawater, at a rate of 4 liters min^{-1}. All tanks were aerated and exposed to 80% solar radiation.

Ammonium $[(NH_4)_2SO_4]$ was added to four of these tanks at a rate of 1 ml min^{-1} (Stambler et al. 1994). Final ammonium concentrations in these tanks were 20 μM and 50 μM. In the ambient treatment tanks ammonium concentrations were the same as in Kaneohe Bay surface waters, ca. 2 μM.

In the last two tanks, seawater entered after running through a tank filled with the macroalga *Gracilaria salicornia* (C. Agardh) Dawson. These tanks represented the "stripped" nutrients treatment, with <2 μM ammonium in the incoming water.

Nine nubbins of *P. damicornis*, representing three different colonies, were placed in each of the four nutrient treatments. Nine nubbins of *M. verrucosa* were placed in each of the four nutrient treatments, but were kept in different tanks than the nubbins of *P. damicornis*. All nubbins remained in the tanks 21 days. From these nubbins, six from each treatment were used at the end of the experiment for pigment and tissue analyses.

Analytical Methods

At the end of the experiments, the following parameters were determined:

(1) Respiration rates of the nubbins. For each trial, three nubbins from a single parent colony were placed in a 2.5-liter respiration chamber at night. PVC holders had been cleaned of algal films before respiration measurements. Respiration chambers were placed in a tank 0.5 m deep with running seawater, maintaining the temperature in the chambers at $\approx$26°C. Water within the chambers was continually stirred with a magnetic spin bar. Oxygen concentrations were measured with an oxygen probe (Nestor). Incubations lasted 20–30 min, and the rate of oxygen depletion per unit of surface area was calculated for four to seven sets of nubbins.

(2) Density of the zooxanthellae within

TABLE 1

EFFECT OF AMMONIUM CONCENTRATION ON RESPIRATION RATES (μmols O_2 min^{-1} cm^{-2}) OF NUBBINS (MEAN $\pm$ SD)

AMMONIUM TREATMENT	*Pocillopora damicornis*	*Montipora verrucosa*
Stripped	-0.034 ± 0.005 $n = 4$	-0.054 ± 0.008 $n = 5$
Ambient	-0.035 ± 0.007 $n = 4$	-0.024 ± 0.004 $n = 4$
20 μM	-0.037 ± 0.007 $n = 7$	-0.042 ± 0.011 $n = 4$
50 μM	-0.036 ± 0.010 $n = 7$	-0.027 ± 0.004 $n = 4$

TABLE 2

EFFECT OF AMMONIUM CONCENTRATION ON ALGAL DENSITIES (number of cells cm^{-2}) OF NUBBINS (MEAN ± SD)

AMMONIUM TREATMENT	*Pocillopora damicornis*	*Montipora verrucosa*
Stripped	$1.06 \times 10^6 \pm 2.75 \times 10^5$ $n = 3$	$1.23 \times 10^6 \pm 2.56 \times 10^5$ $n = 3$
Ambient	$1.90 \times 10^6 \pm 5.94 \times 10^5$ $n = 3$	$8.27 \times 10^5 \pm 3.88 \times 10^5$ $n = 3$
20 μM	$1.33 \times 10^6 \pm 2.13 \times 10^5$ $n = 3$	$6.69 \times 10^5 \pm 3.48 \times 10^5$ $n = 2$
50 μM	$2.08 \times 10^6 \pm 3.72 \times 10^5$ $n = 3$	$7.59 \times 10^5 \pm 1.23 \times 10^5$ $n = 2$

their host. Tissue of the nubbins was removed with a jet of water from a high-pressure unit (WaterPik) (Johannes and Wiebe 1970). The volume of the homogenate was determined, and zooxanthellae were counted using a hemacytometer.

(3) Colony surface area. Cleaned skeletons were dipped in warm paraffin wax (Stimson and Kinzie 1991), and the weight of the wax adhering to the skeleton was compared with a series of samples from blocks or plates of cleaned skeletons of known surface area.

(4) Concentration of pigments in the algae and the nubbins. Two methods of extraction of pigments were used. To determine pigments per algal cell, tissue was removed from nubbins, the homogenate was filtered on GFC filters, and the algal pigments were extracted in 90% acetone. To determine pigments per square centimeter, nubbins were placed in a closed beaker with 90% acetone overnight at 4°C. Chlorophyll *a* (chl *a*) and chlorophyll *c* (chl *c*) were measured using the spectrophotometric equations of Jeffrey and Humphrey (1975). Carotenoids were estimated using the method described by Parsons et al. (1984). Concentrations were normalized per zooxanthella and per unit surface area. The Waller-Duncan test (SAS Institute 1987) was then used to compare treatment means.

RESULTS

Nubbins of both species in the stripped, ambient, and 50-μM treatments seemed normal, but nubbins of *M. verrucosa* in the 20-μM treatment appeared to be bleaching. *Pocillopora damicornis* nubbins in the 20-μM treatment appeared normal.

Respiration rates (Table 1) for *M. verrucosa* nubbins in the four ammonium concentrations were highly variable and not statistically distinguishable. There was less

TABLE 3

EFFECT OF AMMONIUM CONCENTRATION ON PIGMENTS (pg) PER CELL (MEAN ± SD)

PIGMENTS PER CELL (pg)	AMMONIUM TREATMENT	*Pocillopora damicornis*	*Montipora verrucosa*
Chl *a*	Stripped	3.12 ± 0.80 $n = 3$	4.51 ± 0.38 $n = 3$
	Ambient	3.05 ± 0.79 $n = 3$	3.61 ± 0.88 $n = 3$
	20 μM	5.94 ± 3.50 $n = 3$	3.36 ± 0.97 $n = 2$
	50 μM	4.67 ± 1.16 $n = 3$	5.65 ± 1.38 $n = 2$
Chl *c*	Stripped	1.08 ± 0.20 $n = 3$	1.88 ± 1.06 $n = 2$
	Ambient	1.37 ± 0.06 $n = 3$	1.53 ± 0.55 $n = 3$
	20 μM	1.70 ± 0.85 $n = 3$	1.50 ± 0.12 $n = 2$
	50 μM	1.40 ± 0.32 $n = 3$	4.00 $n = 1$
Carotenoids	Stripped	0.51 ± 0.27 $n = 3$	0.93 $n = 1$
	Ambient	0.50 ± 0.07 $n = 3$	0.94 ± 0.07 $n = 3$
	20 μM	2.89 ± 1.69 $n = 3$	1.08 $n = 1$
	50 μM	0.67 ± 0.15 $n = 3$	0.74 ± 0.55 $n = 2$

variability in respiration rates in *P. damicornis*, but no significant differences among respiration rates for the four ammonium treatments.

Zooxanthellar densities were not significantly different for *M. verrucosa* in the four treatments (Table 2). Nubbins of *P. damicornis* in the 50-μM treatment had significantly higher zooxanthellar densities than the nubbins in the stripped treatment. For both species, cell densities were about twice the cell densities recorded for nubbins kept on the reef flat in a natural seawater environment (unpubl. data).

Chl *a* and chl *c* per algal cell in *P. damicornis* and *M. verrucosa* did not differ significantly in any of the four ammonium treatments (Table 3). In both species, chlorophyll levels were three to five times lower than those in nubbins kept on the reef flat in a natural seawater environment (unpubl. data). Carotenoids per cell were not significantly higher in the ammonium treatments. The ratio of chl *a* to chl *c* was around 3 in all treatments for *P. damicornis*, but ca. 1 or less for *M. verrucosa*.

Chl *a* concentrations normalized to surface area in *P. damicornis* showed an increase with increasing concentrations of ammonium although this trend was not significant (Figure 1). For *M. verrucosa*, chl *a* cm^{-2} was significantly higher in the 50-μM treatment.

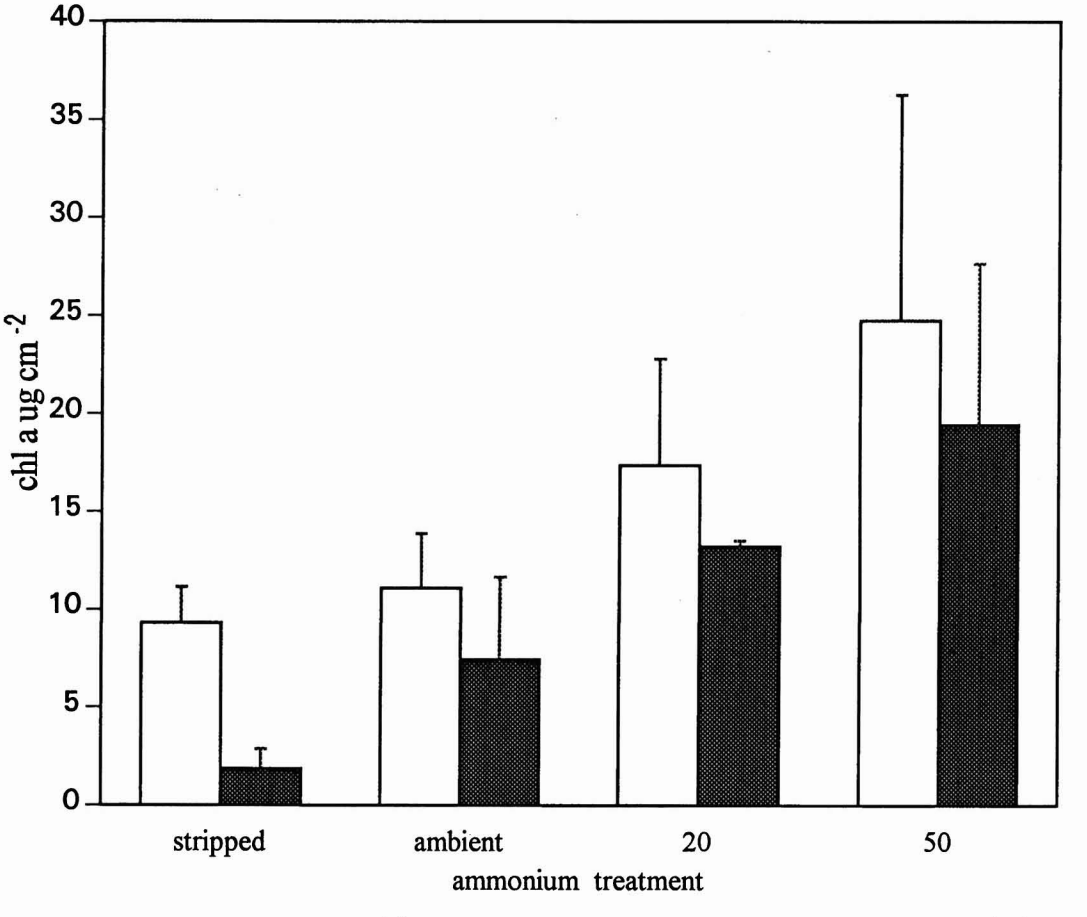

FIGURE 1. Chl *a* concentration (μg cm^{-2}) for *P. damicornis* (open bars) and *M. verrucosa* (solid bars) in the four ammonium treatments (stripped, ambient, 20 μM, and 50 μM).

DISCUSSION

Nitrogen is a limiting factor in oligotrophic seawater, so we expected to find an increase in algal density with increased ammonium concentrations. *Pocillopora damicornis*, but not *Montipora verrucosa*, showed this trend, with highest zooxanthellar densities at the highest ammonium concentration (Table 2). These algal densities for nubbins of *P. damicornis* overlapped values previously reported for intact colonies (Stambler et al. 1991).

In whole colonies of *P. damicornis* and *Stylophora pistillata* Esper, the number of cells per unit surface area increased in response to ammonium enrichment (Muscatine et al. 1989, Dubinsky et al. 1990, Stambler et al. 1991). The response of these experimental nubbins was not as strong as the response of a whole colony. Nubbins may not be representative of the physiology of the whole colony because parts of the colony may respond differently as a result of exposure to different environmental regimes within the colony (Jokiel and Morrissey 1986) or nubbin response may reflect differences in branch age (Titlyanov 1991). Other work with nubbins exposed to elevated ammonium (Høegh-Guldberg and Smith 1989) has demonstrated an increase in algal density in *S. pistillata* but a decrease in algal density in *Seriatopora histrix* Dana.

Ammonium enrichment caused an increase in chl *a* per unit of surface area in both *P. damicornis* and *M. verrucosa* (Table 4, Figure 1). This was also found for *S. pistillata* (Dubinsky et al. 1990), where it represented a combined effect, being the product of increasing algal density and chlorophyll concentration per algal cell. Higher chlorophyll concentrations at high ammonium treatments for *P. damicornis* may have resulted from the trend toward higher zooxanthellar densities and increased chl *a* per cell with enriched ammonium. *Montipora verrucosa* did not increase zooxanthellar densities, but chl *a* per cell showed a trend toward increased pigment at the highest ammonium levels.

Respiration rates of *P. damicornis* and *M. verrucosa* were not affected by the ammonium concentration. Similarly, no significant effect of ammonium concentration was found on respiration rates of entire colonies (Stambler 1992) or nubbins (Høegh-Guldberg and Smith 1989) of *S. pistillata*. Because we cannot separate in vivo the respiration rates of the algae and the animal, and we know that the growth rate of the coral was decreased with ammonium enrichment (Stambler et al. 1991), the lack of differences in respiration rates could be related to an increase in algal respiration, associated with increased algal growth (Falkowski et al. 1985), offset by a decrease in the animal respiration.

The coral-algal association is complex, and disfunction of the symbiosis results from eutrophication (Falkowski et al. 1993). There are demonstrated species-specific responses to light (Kinzie et al. 1984) and nutrient enrichment (Høegh-Guldberg and Smith 1989). In our experiment, although *P. damicornis* re-

TABLE 4

EFFECT OF AMMONIUM CONCENTRATION ON PIGMENTS (μg) PER SQUARE CENTIMETER (MEAN $\pm$ SD)

PIGMENTS PER cm^2 (μg)	AMMONIUM TREATMENT	*Pocillopora damicornis*	*Montipora verrucosa*
Chl *a*	Stripped	9.30 ± 1.83 $n = 3$	1.87 ± 0.99 $n = 3$
	Ambient	11.07 ± 2.80 $n = 3$	7.45 ± 4.19 $n = 3$
	20 μM	17.36 ± 5.44 $n = 3$	13.22 ± 0.28 $n = 2$
	50 μM	24.78 ± 11.48 $n = 3$	19.45 ± 8.23 $n = 3$
Chl *c*	Stripped	2.81 ± 0.58 $n = 3$	5.85 ± 0.89 $n = 3$
	Ambient	3.50 ± 0.83 $n = 3$	13.30 ± 5.35 $n = 3$
	20 μM	4.73 ± 1.59 $n = 3$	11.26 ± 2.20 $n = 2$
	50 μM	6.62 ± 2.69 $n = 3$	11.75 ± 2.10 $n = 3$
Carotenoids	Stripped	5.02 ± 0.63 $n = 3$	5.08 ± 1.50 $n = 3$
	Ambient	6.07 ± 1.46 $n = 3$	6.07 ± 0.57 $n = 3$
	20 μM	8.95 ± 2.63 $n = 3$	13.99 ± 1.83 $n = 2$
	50 μM	12.02 ± 4.47 $n = 3$	16.11 ± 2.74 $n = 3$

sponded to nutrient enrichment with an increase in zooxanthellar density, *M. verrucosa* appeared to show changes in algal pigments rather than zooxanthellar density.

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